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MAZE PERFORMANCE OF THE WHITE RAT IN RELATION TO UNFAVORABLE SALT MIXTURE AND VITAMIN B DEFICIENCY

A DISSERTATION SUBMITTED TO THE
GRADUATE FACULTY IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PSYCHOLOGY

By
MARTIN FREDERICK FRITZ

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MAZE PERFORMANCE OF THE WHITE RAT IN RELATION TO UNFAVORABLE SALT MIXTURE AND VITAMIN B DEFICIENCY[1]

MARTIN F. FRITZ

Iowa State College

INTRODUCTION

Although an enormous amount of work has been done on diets from the physiological standpoint, very few investigations have been concerned specifically with a psychological approach. Miles (3) working with a group of twelve human subjects whose body weights had been reduced 10 per cent and held at that level by limiting the intake of food, found that in general the psychological effects were small. Measurements were made during a period of four months. Stone (25) reports that a degree of inanition in young rats just sufficient to maintain a given body weight results in a delay of the initial copulatory behavior. According to Slonaker (21, 22) when rats are fed a strictly vegetable diet, voluntary activity is so much reduced that it resembles senile conditions. When the rats are returned to a diet drawn from both animal and vegetable sources, voluntary activity again becomes normal. Anderson and Smith (1) found that rats which had been subjected to qualitative and quantitative stunting were superior to normal rats in relearning a maze. In the second relearning following a period of realimentation the records of the experimental and control groups tended toward similarity.

Miss Frank (5) found that rats with active rickets learned the maze in fewer trials and with fewer errors but took much more time than did normal rats. After realimentation the differences in maze learning among normal, rachitic and underweight rats

[1] From the Psychological Laboratory of the University of Chicago. The writer wishes to express his thanks to Professor H. A. Carr who has generously given his time to criticisms of the experiment.

tended to disappear indicating a rather rapid recovery from whatever effects the rachitic diet may have had. In general, she questions the existence of a valid difference in maze learning ability caused by the rickets and suggests that the difference, if present at all, may be too small to be conclusively measured by the maze technique.

The work of Maurer and Tsai (17, 18) bears, perhaps, the closest relationship to the investigation herein reported but several important differences must be noted. These investigators subjected rats to a depletion of the vitamin B complex during the nursing period only, after which a normal diet was fed. In the second place the effect of the depletion upon behavior was tested subsequently at the age of seventy days. Under these conditions they found that the depleted rats showed significantly poorer maze learning records than those on an adequate diet. In the present experiment vitamin depletion did not take place until ninety to one hundred days of age or practically at maturity and occurred simultaneously with the testing of the effects upon behavior. Moreover, the vitamin deficiency was accompanied by an unfavorable salt mixture which also introduced an important dietary difference. It seems that no investigation has been concerned with faulty mineral content of a diet and its effect upon behavior nor with the effect of vitamin deficiency upon the behavior of mature rats following a period of optimum growth and favorable feeding.

STATEMENT OF PROBLEM

It is now recognized that an adequate diet must not only contain proteins, carbohydrates, fats, salts and vitamins but must contain these classes of substances in certain relative proportions, although the first three can be varied considerably for some time without serious consequences. An adequate diet is one which will result in an optimum degree of growth, activity and reproduction through a number of generations. The investigations of McCollum (13) "brought to light the fact that a diet may be sufficiently good to enable an animal to grow in a perfectly normal manner, and to exhibit a moderate amount of fertility, yet

with each succeeding generation the progeny becomes smaller and less well developed physically, and after two, three or four generations the strain dies out."[2] Any diet which results in a deviation from the optimum is inadequate to that extent. Many degrees of inadequacy are possible simply by the variation of one or more of the substances. It is well known that if there is a reduction or an absence of one or more of the vitamins, detrimental physiological effects will occur.

The present experiment is an attempt to relate certain faulty synthetic diets known to have harmful physiological effects, to the maze behavior of practically mature white rats. The following experimental diets were used:

Group II. Unfavorable salt mixture

	per cent
Casein	20.0
Yeast	5.0
Butter	5.0
Bone ash	3.0
$FeSO_4 \cdot 7H_2O$	0.5
NaI	0.5
Dextrin	66.0

Group III. Unfavorable salt mixture and vitamin B deficient

	per cent
Casein	20.0
Butter	5.0
Bone ash	3.0
$FeSO_4 \cdot 7H_2O$	0.5
NaI	0.5
Dextrin	71.0

The group II diet would be considered adequate were it not for the unfavorable salt mixture. The group III diet is identical with the group II diet except for the fact that yeast is withheld and an equivalent weight of dextrin is added. In this way vitamin B[3] is excluded without otherwise materially changing the diet. In both diets the salt mixture is referred to as unfavorable since the minerals are not present in the optimum amounts as compared to successful salt mixtures such as the McCollum No. 185 (14) or that Osborne and Mendel (19).

The amount of iron salt is considerably above the optimum. Starkenstein (23) reports that ferrous salts have a poisonous effect upon rabbits, dogs and frogs. The degree of effect varies

[2] Op. cit., p. 372.

[3] The term vitamin B is used to mean the vitamin B complex now recognized as having a multiple nature.

considerably with the animal, the kind of salt and the amount of salt used. McGuigan (15) found that hypodermic administration of iron is almost invariably followed by toxic effects. Jones (10) suggests that certain unfavorable results found in avitaminosis experiments may have something to do with an overdose of iron. However, iron in small quantites is necessary in order to make an adequate diet.

The iodine content of the diets is also considerably above the optimum. The work of Hesse (7) and Grabfield, Alpers and Prendia (6) indicates that sodium iodide has an effect upon protein breakdown as evidenced by a marked rise in urinary nitrogen. In the case of human beings poisonous effects have been noted. Marshall (16) states that "many people are extremely susceptible to iodides in any form. In them a series of symptoms collectively known as iodism develop when iodides are taken even in small doses" and toxicity is indicated by "cutaneous eruptions, mental depression, and, after continued administration, by loss of flesh and other symptoms." On the whole, the manner in which iodine brings about deleterious symptoms is very little understood at the present time. Iodine in minute quantities is probably a necessary constituent of an adequate diet.

According to available bone analyses the amount of chlorine introduced into the experimental diets by means of the bone ash is considerably under that employed in most synthetic diets which are now thought to be adequate.

It is well established that neural degeneration may follow a deficiency of vitamin B although the effects vary considerably from one individual to another as well as from one species to another. Stern and Findlay (24) reported that young rats fed a diet deficient in vitamin B showed some chromatolytic changes in the ganglion cells of the cord. Vedder (26) has summarized evidence showing that deprivation of vitamin B results in a marked degeneration of nervous tissue. This is of considerable interest, psychologically, since it is generally believed that behavior is intimately related to the nervous system.

Although we have suggested a few of the harmful effects to be expected as a result of the unfavorable salt mixture we cannot

insist too much upon specific effects correlated with the specific components of the salt mixture. McCollum states:

> We have reached the conclusion that it is not possible to state the amount of any food factor which constitutes the minimum or the optimum, or an excess, without taking into consideration the biological values of each of the other factors in the diet. The minimum amount of any dietary factor of constant quality which will just serve to maintain apparently normal well-being, will fail to suffice when another factor is modified so as to be unsatisfactory, and after such a modification of the diet increasing either of the two factors under consideration will suffice to protect the body from harm in some measure. There is a principle of relativity which is of fundamental importance in estimating the value of a diet as a whole. The diet must be considered as made up of a number of factors, and an effort made to adjust each factor at the optimum.[4]

It is clear, then, that faulty dietaries as herein outlined constitute one method of making a severe assault upon the physical organism with a view to observing the effects upon behavior.

EXPERIMENTAL PROCEDURE

The following groups of rats were used:

Group I. Bread and milk—30 rats (15 males and 15 females).
Group II. Unfavorable salt mixture—58 rats (29 males and 29 females).
Group III. Unfavorable salt mixture and vitamin B deficient—60 rats (30 males and 30 females).

Whenever possible litters were divided among the groups.

All of the rats were between ninety and one hundred days of age when started on the problem and first fed the faulty diets. Previous to this time they had been fed liberally on bread and milk with a weekly feeding of kitchen scraps and sunflower seeds. The diets were placed in the food box of the maze and after the daily trial the experimental groups had access to the food for one-half hour. The rats in group I were permitted to eat bread

[4] Op. cit., p. 371.

and milk for ten minutes after each trial. Since feeding did not take place until after the running of the maze the performance on the first day was necessarily uninfluenced by the diets.

One trial per day was given for a period of six weeks. This period of time was uniform for all the animals and no criterion of learning was used. The plan was simply to observe the daily maze behavior of all the rats for six weeks, to note the general trend of each group and to make comparisons between groups.

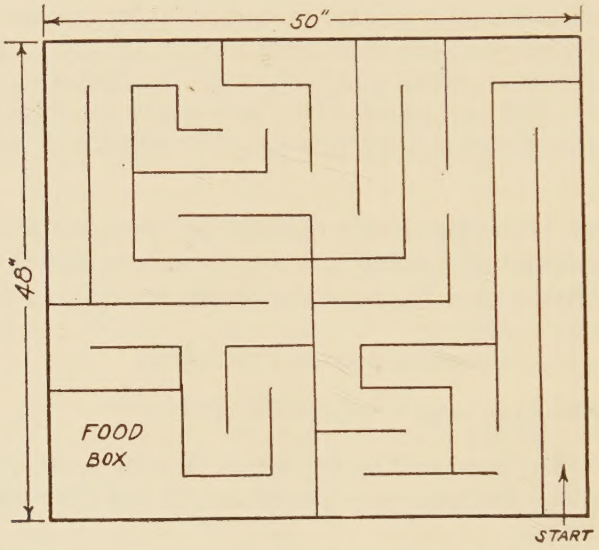

FIG. 1. PLAN OF THE CARR MAZE USED THROUGHOUT THE EXPERIMENT

In general, the experiment may be looked upon as an efficiency of performance test.

Time was recorded to the nearest second. Retraces were counted as one for each straight section of the maze actually backtracked. An error was recorded only in case a rat entered a blind alley with the entire body up to the base of the tail. Any animal placed in the maze and failing to reach the food box in thirty minutes was removed and not fed that day. It was necessary to assume some arbitrary limit such as this since the animals would occasionally be sick and refuse to run.

Figure 1 shows the plan of the Carr maze which was used throughout the experiment. All of the maze except the food box was covered with glass.

The rations were made up about twice a week which would keep the oxidative action of the ferrous sulfate at a minimum, particularly, in respect to its action upon the butter.

RESULTS

Time. Table 1 shows the total time taken by each of the three groups on each of the forty-two days of the experiment. Also, the mean time per rat is shown. It is interesting to note the extremely rapid drop from a relatively high initial time record. On the second day the mean time per rat for all three groups drops to less than half of that on the first day. Again, on the third day there is another drop of about 50 per cent. After the third day the decline in mean time is still pronounced but the percentage reduction is less rapid.

The relationship of the three groups in reference to the mean daily time per rat for the period of forty-two days is shown graphically in figure 2. The values for the first two days are not plotted because it would necessitate compressing the ordinates to such an extent that the relative variability could not be shown clearly. The experimental groups consistently take more time except that on the first day the bread and milk group occupies an intermediate position. After the first day the lowest time values are without an exception in favor of the bread and milk group. Table 4 shows that this difference in time is statistically significant. The difference in time remains about the same in absolute amount except for a brief period from about the eighteenth to the twenty-fifth day.

The special diet groups show considerably more daily fluctuation in time than the bread and milk group. This greater fluctuation is more significant than would seem to be the case at first glance since the experimental groups contain about twice as many animals as the bread and milk group. The mean time values tend to fluctuate less violently with a large group. It is necessary to point out that the deviations were nearly always caused

TABLE 1

Daily records of time

The average time per rat is shown graphically in figure 2.

DAY	TOTAL TIME			MEAN TIME PER RAT		
	Group I	Group II	Group III	Group I	Group II	Group III
	seconds	*seconds*	*seconds*	*seconds*	*seconds*	*seconds*
1	19,518	40,976	34,855	650.6	706.5	580.9
2	7,276	17,851	16,796	242.5	307.7	284.3
3	2,765	7,529	8,839	92.1	134.4	148.1
4	1,936	8,333	6,043	64.5	143.6	100.7
5	1,146	5,257	4,937	38.2	92.2	82.3
6	1,093	6,093	4,328	36.4	105.0	72.1
7	861	5,148	5,338	28.7	88.7	88.9
8	913	3,696	3,168	30.4	64.8	53.6
9	624	3,700	4,909	20.8	63.7	84.6
10	758	3,749	4,860	25.2	65.7	83.7
11	459	4,607	2,269	15.3	80.8	40.5
12	440	5,305	3,128	14.6	91.4	54.8
13	455	3,362	2,713	15.1	63.4	47.5
14	426	5,420	2,668	14.2	96.7	46.8
15	1,120	3,643	2,814	37.3	66.2	49.3
16	575	4,384	1,525	19.1	82.7	27.2
17	626	4,094	2,424	20.8	78.7	44.8
18	608	2,194	1,657	20.2	44.7	30.6
19	504	1,546	1,263	16.8	29.7	22.9
20	748	3,364	2,731	24.9	64.6	51.5
21	462	2,040	1,783	15.4	42.5	34.2
22	758	1,638	1,388	25.2	34.8	26.4
23	1,007	2,062	1,850	33.5	43.8	34.9
24	355	4,783	1,161	12.2	97.4	21.9
25	429	1,384	1,221	14.7	28.8	23.4
26	437	1,695	3,011	14.5	37.6	57.9
27	499	2,774	1,822	16.6	61.6	35.7
28	384	3,503	2,557	12.8	79.6	50.1
29	406	1,365	3,843	13.5	29.6	76.8
30	358	3,023	1,533	11.9	67.1	30.0
31	355	1,181	1,255	11.8	29.5	25.6
32	350	4,854	1,106	11.6	112.8	22.5
33	416	2,567	3,568	13.8	61.1	75.9
34	444	1,728	4,903	14.8	40.1	104.3
35	699	2,651	5,141	23.3	64.6	114.2
36	491	2,021	1,411	16.3	53.1	33.5
37	362	2,078	1,408	12.0	50.6	33.5
38	434	4,107	2,492	14.4	95.5	57.9
39	390	2,436	1,112	13.0	60.9	25.8
40	565	1,341	3,409	18.8	31.2	85.2
41	479	1,824	1,026	15.9	43.4	24.4
42	413	1,710	1,403	13.7	43.3	35.0

by the unusually poor records of a few rats. However, the same animals rarely continued to make a poor record for more than two or three days after which there would be a return to normal running time. Two of the major fluctuations in the graph for group I were due entirely to the poor records of two different animals, one on each occasion. We can only speculate as to the reason for these fluctuations and suggest a temporary loss of appetite as the most likely explanation. The fluctuations in mean daily time in both experimental groups tend to be more violent during the second half than during the first half.

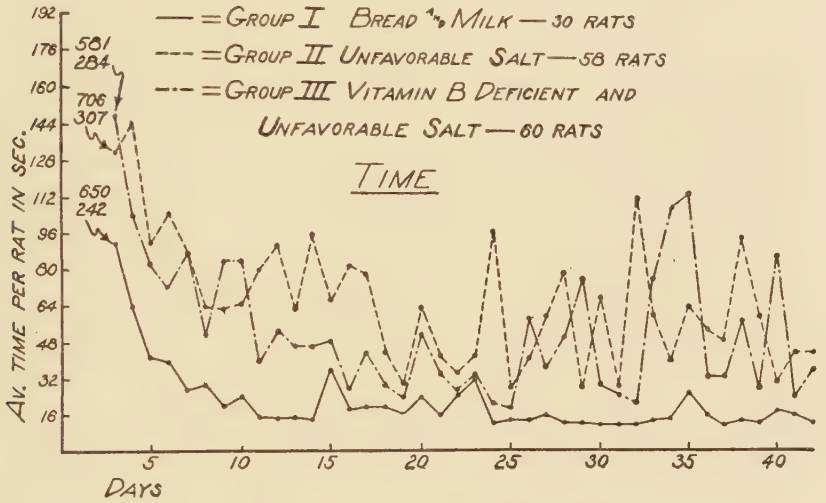

FIG. 2. RELATIONSHIP OF THE THREE GROUPS IN TERMS OF DAILY MEAN TIME PER RAT REQUIRED TO RUN THE MAZE

There is a rather persistent tendency toward a lower mean daily time on the part of group III as compared to group II during the first twenty-five days. After the twenty-fifth day the difference trend is practically lost as a glance at figure 2 will show. Group III has a greater mean time on only nine of the forty-two days but most of these occur during the later stages when the similarity between the two groups is greatest.

To summarize, all groups show an extremely rapid drop in time during the first three days; the special diet groups show consistently

TABLE 2

Daily records of errors

The mean error per rat is shown graphically in figure 3.

DAY	TOTAL NUMBER OF ERRORS			MEAN ERROR PER RAT		
	Group I	Group II	Group III	Group I	Group II	Group III
1	347	605	550	11.5	10.4	9.2
2	190	389	423	6.3	6.7	7.2
3	93	201	262	3.1	3.6	4.4
4	86	227	185	2.8	3.9	3.1
5	59	153	159	2.0	2.7	2.7
6	45	167	145	1.5	2.9	2.4
7	35	144	147	1.1	2.5	2.5
8	33	93	98	1.1	1.6	1.7
9	23	94	119	0.8	1.6	2.1
10	23	99	90	0.8	1.7	1.7
11	18	81	56	0.6	1.4	1.0
12	16	55	50	0.5	0.9	0.9
13	17	63	60	0.6	1.2	1.0
14	15	65	45	0.5	1.2	0.8
15	24	51	45	0.8	0.9	0.8
16	13	59	15	0.4	1.1	0.3
17	13	55	41	0.4	1.1	0.8
18	16	32	32	0.5	0.7	0.6
19	15	11	16	0.5	0.2	0.3
20	20	37	32	0.7	0.7	0.6
21	8	29	29	0.3	0.6	0.6
22	21	21	20	0.7	0.4	0.4
23	39	24	21	1.3	0.5	0.4
24	4	62	13	0.1	1.3	0.2
25	7	13	32	0.2	0.3	0.4
26	7	24	41	0.2	0.5	0.8
27	13	48	29	0.4	1.1	0.6
28	5	37	40	0.2	0.8	0.8
29	6	38	66	0.2	0.8	1.3
30	4	23	24	0.1	0.5	0.5
31	5	18	21	0.2	0.5	0.4
32	4	88	15	0.1	2.0	0.3
33	5	39	60	0.2	1.0	1.3
34	4	24	78	0.1	0.6	1.7
35	9	22	71	0.3	0.6	1.6
36	10	20	16	0.3	0.5	0.5
37	2	14	13	0.1	0.4	0.3
38	4	40	30	0.1	1.0	0.7
39	4	33	6	0.1	0.8	0.1
40	7	13	58	0.2	0.3	1.4
41	3	22	8	0.1	0.6	0.2
42	2	19	15	0.1	0.5	0.4

greater daily time records; this daily difference is statistically significant; the difference in absolute amount of time remains, roughly, the same; the special diet groups show greater daily fluctuation and this daily fluctuation has a tendency to increase during the second half of the experimental period.

Errors. Table 2 shows the total number of errors made by each group and the mean number of errors per rat on each of the forty-two days. The rapid initial drop in mean time to which we have already called attention is closely paralleled by a rapid

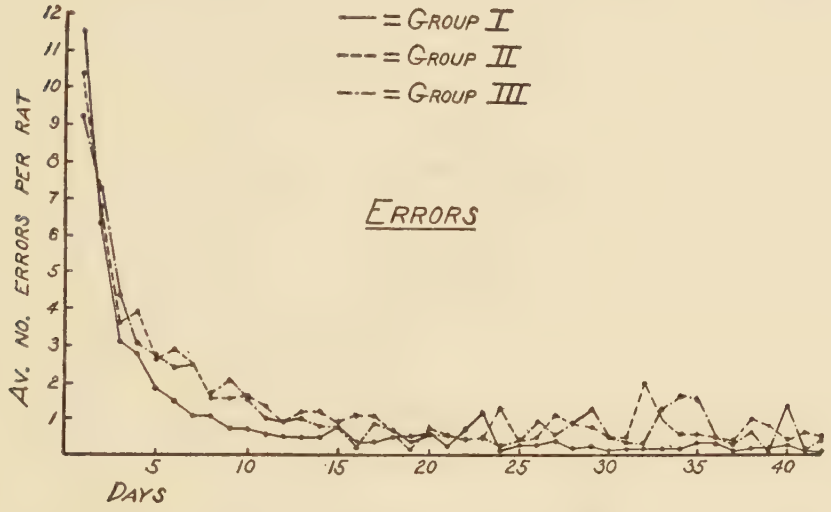

FIG. 3. RELATIONSHIP OF THE THREE GROUPS IN TERMS OF DAILY MEAN NUMBER OF ERRORS PER RAT

initial drop in the mean number of errors per rat. On the second and the third day there is approximately a 50 per cent drop from the record of the previous day after which the decline continues but less rapidly.

Figure 3 is a graphic comparison of the groups in terms of the mean daily error per rat. The special diet groups are characterized by greater daily fluctuations and there is a tendency for these fluctuations to become more pronounced after the twenty-second day. From about the fifth to the fifteenth day the ex-

perimental groups did not reduce errors quite as rapidly as the bread and milk group. In general, it may be said that the error curves of the experimental groups bear a decided resemblance to the error curve of the control group and that the differences do not seem to be large.

In table 4 we see that the X^2 value, following the Chi Square method as outlined by Fisher (4), for the difference between groups I and II is 6.892. If we insist upon a rigid standard and accept 7.824 as a critical value, we can hardly say that the deviation from proportionality is great enough to indicate a definite effect due to diet. The same is true when groups I and III are compared. Opposed to this is the fact that the special diet groups show a *persistent* tendency toward a slightly greater mean daily error.

We may summarize the findings concerning errors and say that there is a rapid initial drop on the part of all three groups; the special diet groups show somewhat greater daily fluctuations which become more pronounced during the second half of the experimental period; from the fifth to the fifteenth day the control group reduced errors more rapidly; the special diet groups have a rather persistent tendency toward a slightly greater error and the curves for all three groups are, in general, very similar. The diets did not cause valid group differences in errors as judged statistically since none of the group differences are large enough to pass the criterion.

Learning—trials. In order to compare the experiment more closely with that of Maurer and Tsai we may apply to the data the same criterion of learning used in their experiment. They used the rather rigid criterion of 8 trials errorless out of 10 and also omitted the first three trials on the grounds that these trials are so largely a matter of chance. Table 5 shows that none of the groups differ significantly so far as number of trials required to learn are concerned. The probable errors of the differences indicate that the group differences are well within the usual statistical limits. Even when the calculations are made by an entirely different method, that of X^2, there is no significant deviation from proportionality.

TABLE 3

Daily records of retraces

DAY	TOTAL NUMBER OF RETRACES			MEAN RETRACE PER RAT		
	Group I	Group II	Group III	Group I	Group II	Group III
1	942	1,473	1,412	31.4	25.4	23.5
2	410	788	855	13.6	13.5	14.4
3	114	341	420	3.8	6.1	7.1
4	115	332	236	3.8	5.7	3.9
5	47	184	184	1.5	3.2	3.0
6	39	292	187	1.3	5.0	3.1
7	28	222	247	0.9	3.8	4.1
8	12	141	143	0.4	2.4	2.4
9	19	152	266	0.6	2.6	4.5
10	25	142	232	0.8	2.5	4.0
11	10	153	90	0.3	2.6	1.6
12	3	93	88	0.1	1.6	1.5
13	10	82	75	0.3	1.5	1.3
14	4	97	66	0.1	1.7	1.1
15	39	96	89	1.3	1.7	1.5
16	11	143	9	0.4	2.7	0.2
17	7	128	73	0.2	2.4	1.3
18	7	49	26	0.2	1.0	0.5
19	15	25	9	0.5	0.5	0.2
20	23	52	76	0.8	1.0	1.4
21	2	67	27	0.1	1.4	0.5
22	17	21	25	0.6	0.4	0.5
23	96	42	32	3.2	0.9	0.6
24	1	151	5	0.0	3.0	0.1
25	5	15	12	0.2	0.3	0.2
26	7	35	103	0.2	0.8	1.9
27	11	117	45	0.4	2.6	0.9
28	3	103	59	0.1	2.3	1.1
29	7	103	142	0.2	2.2	2.8
30	1	71	12	0.0	1.5	0.2
31	3	49	24	0.1	1.2	0.5
32	2	211	11	0.1	4.9	0.2
33	5	126	86	0.2	3.0	1.8
34	5	73	73	0.2	1.7	1.5
35	9	66	109	0.3	1.6	2.4
36	12	69	18	0.4	1.8	0.4
37	0	46	52	0.0	1.1	1.2
38	11	125	67	0.4	3.0	1.5
39	1	96	21	0.0	2.4	0.5
40	26	37	143	0.9	0.9	3.5
41	10	33	33	0.3	0.8	0.8
42	2	63	23	0.1	1.6	0.6

Figure 4 is a graphical analysis of the number of trials required to learn by each of the groups when the sizes of the groups have been adjusted proportionally. This means that groups I and II were raised to a size equal to that of group III by simple arithmetic proportion on the assumption that, had 47 rats reached the criterion of learning, as did in the case of group III, there would still be the same relative distribution as was actually found with the smaller number of rats. It is apparent that great similarity exists among the groups. Although one rather marked deviation

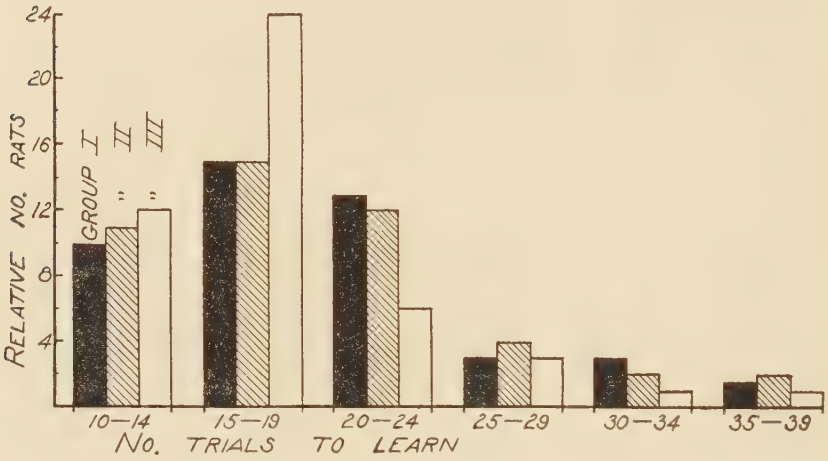

FIG. 4. RELATIVE NUMBER OF RATS IN EACH GROUP MEETING THE CRITERION OF LEARNING IN THE NUMBER OF TRIALS AS INDICATED ON THE BASE LINE

The groups have been equated proportionally so as to rule out the factor of a size difference.

occurs in the case of group III which shows 24 rats learning in 15 to 19 trials inclusive as compared to 15 rats for each of the other two groups, this deviation may be attributed to the operation of chance factors as indicated by the calculations in table 5.

To summarize, it may be said that there is very little evidence of valid group differences which may be attributed to the diets as measured by the number of trials required to learn when the learning criterion is 8 trials errorless out of 10 with the first three trials omitted.

Learning—time, errors and retraces. Table 4 shows the relationship of the groups in terms of time, errors and retraces when no criterion of learning has been applied. Groups II and III differ from group I more than would be expected by chance, statistically speaking, in the case of time and retraces but not in the case of errors. Group differences in the case of time are much greater than corresponding comparisons in terms of either errors or retraces. Table 5 shows the relationship of the groups in terms of trials, time, errors and retraces when the criterion of learning is applied. When tables 4 and 5 are compared it will be seen that there are some differences in the X^2 values. However, whether the criterion of learning is applied or not the deviations from proportionality are in either case greatest in terms

TABLE 4

Comparisons of mean daily performances. Relation of the groups in terms of mean time in seconds, the mean number of errors and the mean number of retraces per rat as shown by the method of X^2

$n = 2$ in each comparison, $P_{0.02} = 7.824$

GROUP	TIME		ERRORS		RETRACES	
	II	III	II	III	II	III
I	47.870	29.613	6.892	3.718	34.744	16.285
II		5.910		0.620		5.437

of time. This, together with the fact that there are no valid group differences in number of trials required to learn, leads to the conclusion that the rate of running the maze was more affected than the precision.

Lethal effects. Thirty-three rats died during the experiment, 15 in group II and 18 in group III. Of this number 25 made a run on the day previous to death. Table 6 shows the high degree of similarity in maze performance between those dying and those not dying in the experimental groups. Comparisons were made by simply taking the record of each rat on the day before death and comparing it with the mean record (for the same day) of all the other rats not dying but which were being fed the same experimental diet. The value of X^2 for errors is 0.8462 ($n = 3$,

TABLE 5

Results in terms of trials, time, errors and retraces when the criterion of learning is 8 trials errorless out of 10

Trials

GROUP	NUMBER OF RATS IN GROUP	NUMBER OF RATS REACHING CRITERION	TOTAL	MEAN	MEDIAN	S.D.	Diff. / P.E.diff.		X^2 $n = 5$ $P_{0.02} = 13.388$	
							II	III	II	III
I	30	28	560	20.0±0.87	19.5±1.1	6.9	0.47	2.23	0.4198	5.154
II	58	46	900	19.5±0.61	19.4±0.89	6.1		0.81		4.918
III	60	47	832	17.7±0.54	17.2±0.80	5.5				

Time

GROUP	TOTAL TIME	MEAN	MEDIAN	X^2 $n = 1$ $P_{0.02} = 5.412$	
				II	III
	seconds				
I	13,880	495	407	14.290	2.606
II	72,962	1,586	941		8.378
III	42,327	900	472		

Errors

GROUP	MEAN	MEDIAN	S.D.	Diff. / P.E.diff.		X^2 $n = 1$ $P_{0.02} = 5.412$	
				II	III	II	III
I	16.9±1.4	14.5±2.4	11.0	4.7	1.0	9.783	0.957
II	30.1±2.5	22.5±4.6	26.2		3.6		6.732
III	19.2±1.7	16.2±3.0	17.4				

Retraces

GROUP	TOTAL	MEAN	MEDIAN	X^2 $n = 1$ $P_{0.02} = 5.412$	
				II	III
I	430	15.3	7.5	6.825	0.627
II	2,311	50.2	31.5		4.821
III	1,459	31.0	9.0		

$P_{0.02}$ = 9.837), which means that the slight deviations actually found are to be considered chance fluctuations. From this it may be concluded that although the experimental diets were severe enough to cause death they had no significant effect upon the maze behavior of those animals dying during the course of the experiment as compared with those which survived on the same experimental diets. In other words, although 33 rats died presumably from the effects of the diet, their records as a group were no worse than those surviving. This finding is of great importance since we might reasonably expect the influence of the diets to be at a maximum on the day previous to death. It may be stated that the records of these 33 rats up until the time of death are included in tables 1, 2 and 3. The only omissions were

TABLE 6

Comparison of those dying and those not dying. Trials on the days previous to death compared with trials on the same day of those surviving

GROUPS II AND III	NUMBER OF COMPARABLE TRIALS	ERRORS		RETRACES		TIME	
		Total	Average per rat	Total	Average per rat	Total	Average per rat
						seconds	*seconds*
Dying	25	15	0.60	15	0.60	1,598	63
Not dying	467	409	0.87	751	1.60	29,250	62

those that necessarily had to be made because of death or refusal to run in the half hour allowed.

Although slightly more deaths occurred in group III than in group II we have no statistical reason for attaching significance to this deviation from proportionality (X^2 = 0.250, n = 1, $P_{0.02}$ = 5.412). This suggests that vitamin B deficiency had a rather small influence, if any at all, in causing the death of the animals. Some possible reasons for this are taken up in the discussion below.

Sex differences. The males and females do not differ significantly in any of the three groups in the number of trials required to learn, total errors, total time or total number of retraces. The X^2 values are 3.770, 0.340, 0.073 and 0.453 respectively (n = 2,

$P_{0.02} = 7.824$). This is in accord with the findings of other investigators who, in general, report but slight sex differences of doubtful significance in maze performance.

While no sex differences were discovered in maze behavior it does seem that there are sex differences in the ability to withstand the lethal effects of the experimental diets since the number of females dying is significantly greater than the number of males dying. Twenty-three females and 10 males failed to survive the six-week period of the experiment. Sex comparisons give a X^2 value of 7.109 ($n = 1$, $P_{0.02} = 5.412$) which, being well above the critical value, indicates a significant deviation from proportionality. We may check this in still another way, viz., by expanding the binominal to the 33rd power. By this method we find that a disproportion of 23 females and 10 males may be expected, roughly, one time in one hundred (1.07 per cent). This agrees with the observations of Dr. V. E. Nelson, physiological chemist at Iowa State College, who has for years been investigating a great variety of diets and with whom this point was discussed. It seems that no one has published specifically on this point.

Practically the same relative proportions of males and females died in the two experimental groups showing that the inadequate diets were equally effective in causing significantly more females to die than males. X^2 for this comparsion is 0.172 ($n = 1$, $P_{0.02} = 5.412$), indicating a great similarity of the experimental groups in this respect.

DISCUSSION

The mean daily time required to run the maze is greater for the experimental groups than for the bread and milk group. This may be explained by assuming that the experimental rats were unable to run rapidly or that they were not adequately motivated or perhaps both. Inability to run rapidly may in turn be due to (*a*) lessened motor ability or (*b*) greater number of errors and retraces. Lessened motor ability does not seem wholly adequate as a single explanation since nearly all of the experimental animals ran the maze on a few trials as rapidly as the

average for the bread and milk group. Some of these trials occurred near the end of the experimental period making it doubtful that motor inability played a very significant part. At least it was not a constant factor.

We have already called attention to tables 2 and 3 which show that the mean number of errors per rat was greater for those animals on the special diets. In table 4 it will be seen that group differences are much greater in the case of time, particularly as compared to errors. This should lead us to suspect that the greater time values are not entirely due to more errors and retraces but rather to an actual slowing up of the rate of running. There is further evidence to support this contention. An examination of the original data shows that in the bread and milk group there were 724 trials (one rat for one day) without errors or retraces. The mean time for these trials was 12.8 seconds. In group II on the unfavorable salt mixture there were 1006 trials without errors or retraces. The mean time was 21.0 seconds. In group III subjected to the unfavorable salt mixture and vitamin B deficiency there were 1167 trials without errors or retraces and the mean time for these trials was 19.1 seconds. Clearly, the animals on the special diets ran much more slowly in those trials where errors and retraces were not involved.

The greater error and retrace records of the experimental groups do not necessarily indicate an inability to solve the maze problem. It is well known that faulty diets frequently have a marked effect upon the hunger behavior of rats. Those animals not motivated to make the trip in the shortest possible time might conceivably engage in leisurely explorations of the maze which would cause an increase in errors and retraces as well as a very marked increase in time. The evidence seems, then, to favor reduced motivation as the most likely explanation of the high time values obtained in the case of the experimental groups.

It is interesting to note that this slowing up in the speed of running is similar to that found in other experiments. As an example, we may mention Arlitt's (2) work concerning the effect of alcohol on the maze behavior of white rats. She found that "alcohol exerts its most pronounced effect on total time" and that

"the increase is not due to alcoholics taking a longer path"[5] but to an actual slowing up in the rate of running. Incidentally, the maze used in the present study on diets is the same one used by Arlitt.

The lack of statistically valid group differences in terms of number of trials required to learn, the indication that time is more affected than precision and the finding that those animals failing to survive the experiment do not differ significantly from the others on the same experimental diets points to the general conclusion that although there was, without a doubt, a very severe attack upon the physiological organism, the behavioral ability, i.e., the capacity to solve the maze problem, was only slightly affected. Three explanations are to be offered which may in part account for this general finding. First, in the case of adult rats the factor of storage may be important. Osborne and Mendel (20) investigated diet in relation to vitamin B content of the liver and state "the conclusion seems inevitable that when an adequate supply of vitamine B is lacking in the diet the store of this factor in the liver tissue, where it is ordinarily found in abundance, becomes largely depleted." Since the rats used in this experiment were not started until they had attained an age of ninety to one hundred days following a period of optimum feeding, there was ample opportunity for storage in the liver. There is also the possibility that the resistance and hardiness of older animals may be due, in part, to a much greater amount of somatic tissue which may be drawn upon in times of need to support the more vital functions.

Second, there is a suggestion that under certain circumstances the vital organs of the body are protected to a certain degree. Lusk (12) says that "long ago Voit showed that the muscles of a cat which starved thirteen days lost 30 per cent while heart, brain and cord lost 3 per cent only."[6] There is, then, the possibility that in mature rats the nervous system is afforded a certain amount of protection so that a breakdown in other parts of the physiological organism resulting in death will occur before

[5] Op. cit., p. 25.
[6] Op. cit., p. 112.

there are marked effects upon the nervous system. Such neural effects may be expected to reflect themselves in behavior. In this connection it is necessary to consider the work of Maurer and Tsai who report that depletion of vitamin B through the mother's milk during the nursing period only has a significant effect upon subsequent maze learning ability. Since the rat is born in a state of relative immaturity much physical development of importance takes place during the first few weeks following birth. Vitamin deficiency during this period in the life of the rat may be serious because of its interference with the normal formative processes. However, after the physiological structures are completed and matured the possibilities of injury may be reduced. It should be remembered that an unfavorable salt mixture and vitamin deficient diet constitutes a double assault upon the organism and in spite of this the effect upon maze ability is apparently much less than that found by Maurer and Tsai. We may conclude that age, which in turn means certain physiological differences, is probably a factor in the amount of disturbance in behavior to be expected as a result of dietary deficiencies.

Third, it may be that although the nervous system is subject to degeneration, only a part of the fibers are needed to carry on the adjustmental behavior as required in the maze situation. In support of this we may quote Vedder (26) who in discussing nerve degeneration due to avitaminosis says:

> Vedder and Clark found that only a relatively small percentage of the nerve fibers were completely degenerated, i.e., axis cylinder destroyed, although the medullary sheath of practically every fiber might show more or less degeneration. It seems probable that the animal is able to do without this small percentage (approximately 10 to 15 per cent) of completely degenerated fibers, so long as the remaining majority of the fibers are capable of transmitting impulses.[7]

There seems to be very little histological evidence that the cerebral tissue is involved in the degenerative processes. Hughes, Aubel and Lienhardt (9) found that lack of vitamin A in the ration of swine resulted in definite degeneration of peripheral nerves and

[7] Op. cit., p. 306.

certain areas of the spinal cord but they specifically assert that they were unable to find degenerative changes in the cerebral tissue. Failure to find degeneration up to the present time may be due to inadequate technique while, on the other hand, it may be another evidence of protective action referred to above. Even if we assume cortical effects the obtained results may still be valid since "it is generally recognized that a slow destruction of the cerebral cortex may be accompanied by almost no symptoms, whereas sudden destruction of the same areas produces marked disturbance of behavior" according to Lashley (11).[8] Coupled with this we may have "a reorganization of the entire neural mass"[9] or a constant readjustment of the nervous system made possible by the daily running of the maze.

Lashley experimentally produced lesions in the spinal cords of rats and found no effect upon accuracy in running the maze. He writes:

> In one set of animals, the pyramidal tracts were completely cut; in another, the rubrospinal; and, in the series of cases, all possible descending tracts were interrupted. All these animals ran the maze in retention tests without errors. They showed marked incoördinations, greater difficulty in negotiating the turns of the maze than did any of the cases with cerebral lesions, but absolutely no disturbance of orientation in the maze.[10]

Evidence is available, then, that the neural degeneration which we may assume took place may have been to a large extent compensated for by a constant readjusting process brought about by the daily training on the maze problem. This would account, in part, for the great similarity between the two experimental groups.

Certain comparisons may be made with the experiment of Miss Frank. She subjected rats to a rickets producing diet at the age of thirty to thirty-five days. The rachitic rats required much more time to learn a maze than did the normal animals and "the increase in time was out of proportion to the amount of motor

[8] Op. cit., p. 103.
[9] Op. cit., p. 25.
[10] Op. cit., p. 115.

involvement produced by the rachitic syndrome." The great increase in time required to run the maze is in agreement with the findings reported in this paper. There is agreement again in that both investigations show fewer trials to learn the maze on the part of the experimental animals, although in the present experiment this advantage was only slightly in favor of the special diet groups. Errors were fewer than for the normal rats while in the present experiment they were somewhat greater. Although there were important differences in age and dietaries the two experiments seem to confirm each other in failing to find deleterious effects on maze ability, except for time, in spite of undoubted harmful physiological consequences. Whether or not effects on behavior can be detected by other techniques still remains to be investigated.

It is generally recognized that the physiological effects of avitaminosis are much less severe in the case of mature rats than with young animals. This would account for the general similarity of the two groups fed the special diets, since the rats used were practically mature, and the observed effects may, perhaps, be largely attributed to the very unfavorable mineral content of the diets.

SUMMARY

Three groups of white rats totalling 148 animals, age ninety to one hundred days, were given one trial per day on a maze for a period of forty-two days. Group I was fed bread and milk, group II a synthetic diet faulty in content and proportion of minerals, and group III a synthetic diet having the same composition as the diet for group II with the added fault of a vitamin B deficiency. The results may be summarized as follows:

1. There are marked individual differences in the ability to withstand the effects of faulty diets.

2. The experimental groups on the faulty diets show considerably more daily fluctuation in mean time and mean errors than the bread and milk group, although it should be pointed out that the deviations were usually caused by unusually poor records of a few rats.

3. There is a tendency for the daily fluctuations in mean time and mean errors of the special diet groups to become somewhat more pronounced during the second half of the experimental period.

4. The experimental groups as compared to the bread and milk group show consistently greater daily time, retrace and error records.

5. In the case of mean daily time and mean daily retraces the experimental groups differ from the bread and milk group by an amount which would be considered statistically significant. This is not true in the case of mean daily number of errors.

6. Since the most significant differences are in terms of time, it would seem that the rate of running is more adversely affected than the precision of performance.

7. Although group differences in terms of errors are not statistically significant, the experimental groups show a rather consistent tendency toward a greater mean daily error.

8. There is no evidence of valid group differences in terms of number of trials required to learn when the learning criterion is 8 trials errorless out of 10 with the first 3 runs omitted.

9. Those rats that died and were able to run the maze on the day previous to death, did not differ significantly in performance from those not dying in the same group.

10. The relative number of rats dying while on the unfavorable salt diet did not differ significantly from the number dying while on an unfavorable salt plus vitamin B deficient diet.

11. The number of females dying is significantly greater than the number of males dying.

12. Both diets were equally effective in causing significantly more females to die than males.

13. There was no evidence of a breakdown in performance during the six weeks of the experiment.

14. This study indicates that relatively mature white rats are able to make daily adjustments to a complicated maze situation with a high degree of accuracy even though subjected to extremely unfavorable diets.

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